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— SECTION C —

## BOTANICAL SCIENCES

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# Canadian Journal of Research

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## THE METABOLISM OF SCOPOLETIN BY HEALTHY AND VIRUS INFECTED POTATO TUBERS[1]


BY SHIRLEY R. ANDREAE[2] AND W. A. ANDREAE[3]



### Abstract

After extraction of the chlorophyll, rolled leaves of potato plants infected with the leaf roll virus fluoresced a brilliant blue color in ultraviolet light. This blue fluorescence was not observed in leaves of healthy potato plants or plants infected with mild mosaic, rugose mosaic, or spindle tuber viruses. Neither was it found in unrolled leaves of leaf roll diseased plants. The blue fluorescent substance in rolled leaves had the same fluorescent and solubility properties as scopoletin.

Scopoletin was metabolized by healthy and diseased potato tubers with the production of an unstable blue intermediary that turned bright yellow on standing. This reaction was accelerated by hydrogen peroxide and inhibited by heating the tissue to 65° C., by cyanide, azide, hydroxylamine, and hydrogen sulphide. Healthy tubers were found to metabolize scopoletin more rapidly than leaf roll infected tubers.


### Introduction

Scopoletin, 7-hydroxy-6-methoxy-1 : 2-benzopyrone, has been identified as a normal constituent of *Scopolia japonica*, *Gelsemium sempervirens*, and a number of other plants (3). In ultraviolet light it shows a bright blue fluorescence similar to that of unbelliferone, a related coumarin.

In 1936 Best (4) working with tobacco plants infected with tomato spotted wilt virus noticed that in ultraviolet light the necrotic lesions were surrounded by blue fluorescent halos. A similar phenomenon was observed in infected potato, petunia, and *Nicotiana glutinosa* plants. Incipient lesions could be detected by fluorescent spots, the centers of which became necrotic within a few days. As the disease progressed the necrotic lesions and their halos spread outwards until they reached another lesion or a vein. The fluorescence then travelled along a lateral vein to the midrib and thence to the petiole and roots. This fluorescence appeared to be associated with necrosis as it was not seen in plants where infection with tomato spotted wilt did not result in necrotic lesions, e.g. in nasturtium plants infected with a mild strain of the virus. Primary lesions of tobacco mosaic virus on *Nicotiana glutinosa* plants showed relatively faint fluorescent halos; these were much less intense than those caused by the spotted wilt virus.


[1] *Manuscript received December 15, 1948.*

*Contribution from the Department of Biology, University of New Brunswick, Fredericton, N.B., with financial assistance from the National Research Council of Canada.*



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In 1944 Best (5) isolated the fluorescent substance from tobacco plants infected with tomato spotted wilt and showed that this substance was scopoletin. He also isolated it from healthy tobacco plants where it occurred in very small amounts, chiefly in the endodermis (4, 6).

It had been reported in the literature (1, 9, 12) that leaf roll virus infected potato tubers that showed phloem necrosis* also showed, when viewed in ultraviolet light, a bright blue fluorescence in the necrotic areas. In 1948 the substance causing this fluorescence was isolated by Andreae (2) and it also was found to be scopoletin. It was not detected in healthy tubers or in infected tubers that did not show phloem necrosis.

The cause of the abnormal fluorescence on virus infection has not been determined. The fact that mechanical injury to tobacco plants resulted in localized patches of slightly enhanced fluorescence suggested to Best (4) that the reaction was not specific for virus infection but had a wider significance in connection with injury to the tissues. Best considered that in the healthy plant scopoletin might be concerned with growth, as topping favored the accumulation of this substance in the roots (4, 5). Theories as to how scopoletin could be produced by the plant are discussed by Best (6) in a recent paper.

Our present work has been limited to a study of conditions that result in the appearance of scopoletin in potato plants and to its metabolism by healthy and leaf roll virus infected tubers.

## Results

### *A. On the Occurrence of Scopoletin in Potato Plants*

Although the fluorescence associated with phloem necrosis in leaf roll diseased tubers had been observed previously, there were no reports concerning any abnormal fluorescence in the leaves of the infected potato plants. The fluorescence of leaves of healthy and of leaf roll diseased potato plants (varieties Green Mountain, Katahdin, Chippewa, Sebago, and Houma) was, therefore, investigated. At the same time Green Mountain potato plants infected with mild mosaic, rugose mosaic, and spindle tuber viruses were also examined for fluorescence. All plant material was obtained from the experimental plots of the Laboratory of Plant Pathology, Fredericton, N.B. The diseased plants were grown from infected tubers and were six to eight weeks old at the time of study. Some thousands of leaves were examined in the course of time. In ultraviolet light the only fluorescence visible in all healthy and infected leaves was the reddish one characteristic of chlorophyll. When, however, the chlorophyll was removed by immersing the leaves in boiling water and then in hot alcohol for a few minutes, the rolled leaves of the leaf roll

* *Phloem necrosis is found only in tubers with primary leaf roll infection i.e. an infection contracted during the current growing season. It occurs only in certain potato varieties (8) and its appearance is influenced by storage conditions (7). Phloem necrosis in the aerial parts is constantly associated with both primary and secondary infections.*

infected plants always were found to fluoresce a brilliant blue color in ultraviolet light. Strongly rolled leaves showed a fluorescence over the entire blade, while slightly rolled leaves fluoresced generally only at their tips. This fluorescence was never observed in healthy leaves or in leaves infected with either mild mosaic, rugose mosaic, or spindle tuber viruses. Nor was it seen in unrolled leaves of leaf roll infected plants.

An attempt was made to see whether fluorescence also developed in the leaves of plants with primary leaf roll. For this purpose leaf roll infected aphids were transferred to healthy Green Mountain plants and allowed to feed on them for five days. After several weeks a slight rolling of the upper leaves of some of the exposed plants became noticeable. These upper leaves also showed a definite starch accumulation and a brilliant blue fluorescence, while the lower, unrolled leaves of the same plants showed neither.

Twenty-five years ago, Murphy (10) showed that an excess of starch in the rolled leaves is a constant feature of potato leaf roll and he concluded from his studies that the rolling of the leaves resulted from the abnormal starch accumulation. Since it was found that fluorescence in the diseased leaves appeared to be associated with rolling, it seemed likely that there might be some relationship between the abnormal starch content and the fluorescence of leaves. Examination of healthy and of diseased, rolled leaves gathered early in the morning bore out this supposition; healthy leaves showed neither starch nor fluorescence, while rolled, infected leaves showed both, and further, the starch appeared only in the portion of the blade that fluoresced. In almost every case, the areas of starch accumulation and fluorescence coincided exactly.

The blue fluorescent substance was isolated from the leaves by the same method that was used for the extraction of scopoletin from tubers. This leaf substance showed the same solubility and fluorescent properties as scopoletin.

*B. On the Metabolism of Scopoletin by Potato Tubers*

When a slice of healthy or leaf roll diseased tuber was allowed to stand in a 10 mgm. % solution of scopoletin at room temperature, some of the scopoletin was found to be taken up by the tissue so that it became fluorescent, but in time there was a loss of fluorescence from both tissue and solution, indicating that scopoletin was being destroyed. Accompanying the loss of fluorescence were certain color changes on the surface of the tissue. The first color change consisted of the appearance of dark blue spots after the tissue had been in the solution for about four hours. These generally occurred first in the vascular regions but ultimately the whole surface became discolored. After about a day the dark blue spots turned yellow.

The addition of a drop of hydrogen peroxide to the scopoletin solution greatly accelerated the rate of loss of fluorescence and the color changes, the whole surface of the tissue turning a uniform dark blue color within a few minutes. The blue substance diffused into the solution so that both tissue and solution became colored. After the development of the blue color was

complete, the tissue and solution turned to a greenish hue and finally to a bright yellow color. The whole process required about three hours for completion. Identical color reactions occurred when scopoletin and hydrogen peroxide were added to potato juice, but they took place even more rapidly and were completed within an hour at room temperature. Potato juice to which only hydrogen peroxide or scopoletin was added showed no similar color changes over a period of 30 hr., indicating that both substances are required for the reaction with juice. Scopoletin is quite stable at room temperature for this length of time, provided it is not exposed to bright light. The juice was prepared by pulping two small potatoes in 100 ml. of water in a Waring blendor and then filtering off the pulp. The juice was not kept for more than two hours. The addition of 2 ml. of a 10 mgm. % solution of scopoletin and one drop of 3% hydrogen peroxide to 2 ml. of the juice caused the rapid development of the blue color, which then gradually changed to yellow. The formation of the blue color was completely inhibited by heating the juice to 65° C. for 15 min., by sodium cyanide (0.001 *M*), sodium azide (0.001 *M*), sodium sulphite (0.01 *M*), hydroxylamine (0.01 *M*), ascorbic acid (75 mgm. %), very small amounts of hydrogen sulphide, and sodium fluoride (1 *M*). Sodium fluoride in a concentration of 0.01 *M*, however, had no appreciable effect on the reaction. The blue color, once formed, could be turned yellow immediately by heating, or by the addition of cyanide, sodium sulphite, ascorbic acid, alkali, or alcohol. No means was found of inhibiting the change from blue to yellow.

The yellow substance appeared to be quite stable at room temperature and was isolated by the following procedure. One hundred milligrams of scopoletin was dissolved in about 5 ml. of hot alcohol and poured into 500 ml. of potato juice. The addition of 2 ml. of 3% hydrogen peroxide turned the solution an inky blue color. On standing at 38° C. for about 15 min. the solution became greenish-yellow. Two milliliters more of hydrogen peroxide was then added upon which the color again became an inky blue. This procedure was repeated until the addition of hydrogen peroxide to the greenish-yellow solution did not cause further appearance of the blue color. The juice was then allowed to stand until the color changed from greenish-yellow to yellow. On adjusting the pH to 4, a bulky yellowish precipitate settled out. This was centrifuged down and the colorless supernatant fluid discarded. After being dissolved in alkaline water (pH 8) and reprecipitated at pH 4, the yellow substance was separated from the bulky precipitate by being shaken with 200 ml. of alkaline alcohol (3 ml. of concentrated ammonium hydroxide per liter of alcohol). This extraction was repeated five times. The alcohol extract was concentrated to about 500 ml. under reduced pressure and passed through an alumina adsorption column. The yellow substance was strongly adsorbed at the top of the column. The column was rinsed with 200 ml. of alcohol and the yellow substance eluted by an alkaline solution (pH 10) of five parts of alcohol to one part of water. The 300 ml. of eluate were concentrated to 20 ml. under reduced pressure. On adjusting the concentrate to pH 4, the

substance settled out in a pale yellow, noncrystalline state. In alkaline solution, this substance was bright yellow and showed a brilliant yellow fluorescence in ultraviolet light. Work is in progress on its identification.

Preliminary studies indicated that the fluorescence of scopoletin solutions was destroyed more rapidly by healthy tuber tissue than by leaf roll infected tissue. Two slightly different procedures were employed to study this effect. By Method A, duplicate cylinders 1 cm. in length and 6 mm. in diameter were punched out of the heel end of a Green Mountain tuber. After being rinsed momentarily in water, the duplicate cylinders were placed in a large culture tube containing 5 ml. of a 0.075 mgm. % solution of scopoletin. One-half milliliter of a 4% solution of sodium fluoride was added to the tube to inhibit bacterial growth. The cylinders were incubated in the scopoletin–fluoride solution at 38° C. for 24 hr. They were then removed from the solution, rinsed, placed in small individual tubes, and the intensity of their fluorescence in ultraviolet light was estimated visually. The intensity of the fluorescence of the scopoletin solution in which the cylinders had been incubated was also estimated. By this method cylinders from 36 tubers and the solutions in which they had been incubated were separated on the basis of their relative fluorescence into three groups viz. strongly, moderately, and slightly fluorescent. An eye of each tuber studied was planted and the plants were inspected for symptoms of leaf roll after eight weeks of growth. The classification of the tubers as healthy or diseased was based on these indexing results.

Table I shows the number of cylinders from the 36 tubers with a strong, moderate, or slight fluorescence after incubation in a 0.075 mgm. % solution, of scopoletin for 24 hr., and the relation of the intensity of the fluorescence to

TABLE I

| | Fluorescence of cylinders* | | |
|---|---|---|---|
| | Strong | Moderate | Slight |
| No. of healthy cylinders | — | 3 | 17 |
| No. of diseased cylinders | 12 | 1 | 3 |

** The results are given for only one cylinder from each tuber as duplicate cylinders showed the same fluorescence.*

healthy and leaf roll virus diseased tissue. Table II shows the relative fluorescence of the scopoletin solutions after incubation for 24 hr. with the above cylinders. These two tables indicate that in slightly over 80% of the tubers studied the rate of loss of fluorescence from both tissue and solution was markedly greater with healthy material than with leaf roll diseased material.

In Method B similar cylinders were taken in duplicate from both the eye and heel ends of each tuber. Duplicate cylinders were placed in 5 ml. of a 0.1 mgm. % solution of scopoletin in a large culture tube and incubated for two

TABLE II

| | Fluorescence of solutions | | |
|---|---|---|---|
| | Strong | Moderate | Slight |
| No. of solutions incubated with healthy cylinders | 3 | 1 | 16 |
| No. of solutions incubated with diseased cylinders | 13 | — | 3 |

hours at 38° C. The cylinders were then removed, rinsed in water, placed in a dish with small partitions, and covered with water. They were incubated for a further two hours and then each cylinder was placed in a small test tube and the intensity of its fluorescence in ultraviolet light was estimated visually. By this method cylinders from 85 tubers were separated into three groups depending on their relative fluorescence. Each of the 85 tubers studied was indexed in the greenhouse and the classification of the tubers was based on the indexing results.

Table III shows the relative fluorescence of the cylinders after the above treatment and the relation of the intensity of the fluorescence to the healthy and diseased tissue. These results indicate that there was a markedly greater loss of fluorescence from the healthy cylinders than from the infected cylinders in 85% of the cases.

TABLE III

| | Fluorescence of cylinders* | | |
|---|---|---|---|
| | Strong | Moderate | Slight |
| No. of healthy cylinders | 5 | 1 | 38 |
| No. of diseased cylinders | 35 | 3 | 3 |

* *The results are given for only one cylinder from each tuber as duplicate cylinders showed the same fluorescence.*

In both methods duplicate cylinders gave the same readings. Cylinders from eye and heel ends also agreed. The groupings obtained by two different workers were almost always identical. The cylinders could be readily separated with a little experience; the liquids were sometimes more difficult to judge. The concentration of scopoletin used in these two methods was too low to cause any appreciable color change on the tissue surface.

## Discussion

It is not known whether scopoletin is a metabolic intermediary in healthy potato plants. The fact that it has been isolated from a number of healthy plants indicates that in some species at least it is a normal metabolic product.

In potato plants, however, it is possible that it may be produced only under certain conditions, of which but two, namely infection with the leaf roll and the tomato spotted wilt viruses, are known at the moment. As infection with mild mosaic, rugose mosaic, and spindle tuber viruses did not cause the appearance of a blue fluorescence in the leaves, this effect does not seem to be a property of viruses in general.

Whether or not scopoletin is a normal metabolite of potato plants, the results show that it can be destroyed by tuber tissue with the production of colored compounds. As the destruction of this substance by the tubers is accelerated by hydrogen peroxide and inhibited by heating the tissue to 65° C., and by low concentrations of cyanide, azide, hydroxylamine, and hydrogen sulphide, a thermolabile peroxidase would appear to be involved. It should be mentioned here that the addition of potassium permanganate to a scopoletin solution causes identical color changes. However, the yellow substance so formed differs from that produced enzymatically in that the former will turn blue again on acidification, while the latter only turns a paler yellow.

If scopoletin is a normal metabolic intermediary in potato plants, its accumulation on infection with the leaf roll virus may be simply ascribed to the decreased ability of the plant to metabolize it so that it is not removed as rapidly as it is formed. Although we do not know why leaf roll infection causes the decreased ability of the plant to metabolize scopoletin, there are several possibilities that at present are being investigated. First, the virus might cause an underproduction of the enzyme or enzymes involved, or the production of them in a less active form. Second, the infection might result in the elaboration of inhibitory agents. Smith and Paterson (13) and Newton (11) have reported a high concentration of ascorbic acid in leaf roll infected potatoes. As we have seen, ascorbic acid in sufficient concentration will inhibit the destruction of scopoletin by tubers. However, more data must be obtained on the actual concentration of ascorbic acid in infected tubers before it can be decided whether or not it is high enough to account for the markedly decreased rate of scopoletin metabolism.

The fact that starch and scopoletin both first accumulate in the tip of the blade suggests that the virus may invade the blade from the tip and from there spread throughout it, causing a more or less general metabolic disturbance in the invaded areas. However, there is as yet no adequate evidence that the starch accumulation is due to a disordered metabolism of this substance, and other factors, such as permeability or translocation abnormalities that could cause a starch accumulation, must not be neglected.

The question arises as to whether any of the fluorescent or metabolic properties of leaf roll infected potato plants can be utilized in testing for infection. As far as the fluorescence in the leaves is concerned, this can be dismissed as being no more useful a criterion of a diseased condition than the presence of an abnormal amount of starch, for under certain conditions of growth, neither starch accumulation nor fluorescence is found in infected plants. Plants in which starch and scopoletin accumulate usually show an

obvious rolling of the leaves and other external symptoms of leaf roll, so that there seems little point in examining their leaves for fluorescence except in doubtful cases where it is not known whether the rolling is of parasitic nature.

A more important consideration is a biochemical test for leaf roll infection in the tubers as at the moment no satisfactory one exists. Newton (11) has published a method for the diagnosis of leaf roll in tubers that is based on their ascorbic acid content, but more must be known about the specificity of such a procedure before it can be accepted. The difference in the rate of scopoletin metabolism between healthy and leaf roll infected tubers also seems to offer some promise for a biochemical test, but as yet sufficient work has not been done on tubers infected with leaf roll and with other virus diseases to determine its specificity.

## Acknowledgment

The authors are much indebted to Dr. D. J. MacLeod of the Plant Pathology Laboratory, Fredericton, N.B., for plant material and greenhouse space. They would like to acknowledge their gratitude to Dr. Léo Marion of the National Research Council of Canada for his kindness in supplying a large sample of scopoletin. This sample was isolated from the roots of *Gelsemium sempervirens* and showed an identical melting point and absorption spectrum with the sample isolated in this laboratory from phloem necrotic potato tubers.

## References

1. ALLAN, J. A. Fluorescence in ultra-violet light as a test for the presence of leaf roll virus in potato tubers. Nature, 155 : 116. 1945.
2. ANDREAE, W. A. The isolation of a blue fluorescent compound, scopoletin, from Green Mountain potato tubers infected with leaf roll virus. Can. J. Research, C, 26 : 31–34. 1948.
3. BEILSTEIN, F. K. Beilsteins Handbuch der organischen Chemie. 18 : 99. Julius Springer, Berlin. 1934.
4. BEST, R. J. Studies on a fluorescent substance present in plants. 1. Production of the substance as a result of virus infection and some application of the phenomenon. Australian J. Exptl. Biol. Med. Sci. 14 : 199-213. 1936.
5. BEST, R. J. Studies on a fluorescent substance present in plants. 2. Isolation of the substance in a pure state and its identification as 6-methoxy-7-hydroxy 1 : 2 benzopyrone. Australian J. Exptl. Biol. Med. Sci. 22 : 251-255. 1944.
6. BEST, R. J. Studies on a fluorescent substance present in plants. Part 3. The distribution of scopoletin in tobacco plants and some hypotheses on its part in metabolism. Australian J. Exptl. Biol. Med. Sci. 26 : 223-230. 1948.
7. FOLSOM, D. Leaf roll net necrosis and stem-end browning of potato tubers in relation to temperature and certain other factors. Phytopathology, 36 : 1016-1034. 1946.
8. FOLSOM, D., LIBBY W. C., SIMPSON, G. W., and WYMAN, O. L. Net necrosis of potatoes. Univ. Maine, Coll. Agr. Bull. 246. 1938.
9. MCLEAN, J. G. and KREUTZER, W. A. The determination of virus infections in potato tubers by the use of ultra-violet light. Am. Potato J. 21 : 131-136. 1944.
10. MURPHY, P. A. On the cause of rolling in potato foliage; and on some further insect carriers of the leaf-roll disease. Sci. Proc. Roy. Dublin Soc. 17 : 163-184. 1923.
11. NEWTON, W. An indicator agar for the determination of the relative concentration of ascorbic acid in potato tuber tissue. Can. J. Research, C, 25 : 242-245. 1947.
12. SANFORD, G. B. and GRIMBLE, J. G. Observations on phloem necrosis of potato tubers. Can. J. Research, C, 22 : 162-170. 1944.
13. SMITH, A. M. and PATERSON, W. Y. The study of variety and virus disease infection in tubers of *Solanum tuberosum* by the ascorbic acid test. Biochem. J. 31 : 1992-1999. 1937.

# ROW TREATMENT OF SOIL WITH TETRAMETHYLTHIURAM DISULPHIDE FOR CONTROL OF BLACKROOT OF SUGAR-BEET SEEDLINGS. I. GREENHOUSE TESTS[1]

By A. A. Hildebrand[2], W. E. McKeen[2], and L. W. Koch[2]

## Abstract

The prevention of blackroot of sugar-beet seedlings has been attempted in greenhouse experiments, by row applications of different proprietary compounds to naturally-infested field soils representing three distinct types and differing biologically with respect to associated pathogens.

Segmented seed, var. U.S. 215 × 216, was used in all the tests as was also a standard, commercial fertilizer. In earlier trials, the chemical under test and the fertilizer were added separately to a measured volume of rootrot soil. In later trials, the chemical mixed with the fertilizer was incorporated with the soil by a method approaching more closely that employed in field practice. The two procedures, designated as the precision and the surface application method, respectively, are described in detail. Calculations for the requisite amounts of chemical and fertilizer were made on the basis of sugar-beet rows in the field being spaced 22 in. apart, *i.e.*, 23,760 linear feet of row per acre.

Tetramethylthiuram disulphide (50%, nonwettable), when applied as Arasan or D-419 at the rate of 3 to 4 lb. per acre, consistently proved to be by far the most effective of the chemicals tested, in reducing pre- and postemergence blackroot. Arasan, which was investigated more intensively than any of the other chemicals, was found (*a*) to retain its fungicidal capability after being mixed for as long as 14 months with a commercial fertilizer, (*b*) to be effective in reducing the incidence of the disease in three quite different types of soil, and (*c*) to remain effective in the soil over relatively long periods of time. These beneficial effects are contingent, however, upon correct placement of the chemical, best results having been obtained when the seed germinated in, and the seedlings grew up through, soil impregnated with the Arasan–fertilizer mixture. Up to 21° C., Arasan, at a rate as high as 4 lb. per acre, will, in a moderately wet soil, effectually control the disease without injury to the seedlings. At 27° to 29° C., moderate injury to roots and lower hypocotyl resulted from a 3 lb. per acre application of the chemical and, following a 4 lb. application, root and hypocotyl injury was accentuated, typical foliar symptoms appeared, and mortality of seedlings was appreciable.

Of the other chemicals tested, 2,4,5-trichlorophenyl acetate (Mycotox No. 1), 20% copper trichlorophenate (C-119), 50% 2,4,5-trichlorophenyl chloroacetate (F-800), and a product of undisclosed chemical composition (CCH-358 or 358A) showed encouraging possibilities.

## Introduction

In southwestern Ontario, as in other sugar-beet-growing areas of the mid-continental humid belt, the disease commonly known as blackroot constitutes one of the most important limiting factors in the obtaining of adequate and uniform stands of seedlings. Several fungi, including *Pythium* spp., *Phoma Betae* (Oud.) Frank, *Rhizoctonia Solani* Kuehn, and *Aphanomyces cochlioides*

[1] *Manuscript received December 22, 1948.*

*Contribution No. 974, Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.*

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Drechs., are known to be capable of causing the disease in Ontario.* In a recent comprehensive review of the blackroot situation as it pertains to the humid area in the United States, Coons, Kotila, and Bockstahler (1) state that a differentiation can be made between what they term acute and chronic phases of the disease. According to these authors, the acute stage is manifested by the killing of young plants during germination or within a week or two after emergence from the soil. Seedlings do not always die but many of those that survive the initial attack never fully recover and their main or lateral roots show a diseased condition that is often characteristic of the primary pathogen involved. Thus, various disease aspects may become apparent in the later stages of growth of the plant, such aspects being regarded as manifestations of the chronic phases of blackroot.

The distinctions that have been made between the acute and the chronic phase of blackroot by the above-mentioned investigators are as applicable to the disease as it occurs in Ontario as to that prevalent in many of the sugar-beet-growing areas of the United States. Both phases have been recognized and have been under investigation by the present authors for the past several years. The acute phase, however, has received the greater amount of attention. In the acute stage, both pre- and postemergence damping-off play a role in the failure of stands. Field observations to date have indicated, however, the considerably greater destructive capacity of the postemergence phase. Season after season, a definite sequence has been observed. Just when emergence is approaching its maximum, blackroot makes its appearance. After a period of several days, during which the seedlings are especially susceptible to attack, stands in many instances are either completely destroyed or reduced to the extent that it becomes questionable whether they should be saved even though, following the period of high mortality, the surviving seedlings show evidence of 'coming out' of blackroot.

Although the terms damping-off and blackroot are used almost synonomously, nevertheless the latter is somewhat of a misnomer; for, in many infected seedlings, the roots are not attacked at all. Rather, the hypocotyl is the vulnerable part of the young plant. Thus, strictly speaking, the term 'black hypocotyl' would be more accurately descriptive of the condition of the seedling. In Europe, the term black leg (8) is used to denote the hypocotyl type of damping-off. The hypocotyl becomes infected usually at or near the soil level. Infection spreads rapidly and the hypocotyl is soon killed or reduced to a thin, black thread that is incapable of supporting the seedling in an upright position.

Because seedlings are especially susceptible to attack by parasitic organisms during a certain period in their early development and because, further, the point of attack is fairly localized, it would seem reasonable to suppose that, if the seedlings were afforded additional protection against the pathogen at the

** Aetiological and other phases of the blackroot problem beyond the scope of the present paper will be fully dealt with in a forthcoming publication by the second author.*

right time and place, they might have a greater chance of survival. Accordingly, during the past three years, the present authors have carefully investigated the possibility of modifying or controlling the disease by adding, to the surface layers of infested soil, various of the newer chemicals possessing fungicidal or fungistatic properties. Of the many protectants tested, formulations containing 50% tetramethylthiuram disulphide as the active agent have given the consistently best results and for this reason most of the experiments described below have centered around the use of this chemical. This new line of attack was adopted only after treatment of seed with several of the older and many of the newer proprietary materials had failed to give adequate protection to the seedling.

## Material and Methods

In experiments carried out under ordinary environmental conditions in the greenhouse, standard wooden flats 24 × 12 × 4 in. were employed. In tests requiring more precise control of environmental conditions, Wisconsin temperature tanks were used. In all experiments the soil was watered moderately as required. Many of the tests were carried out during the winter months in a heated greenhouse, where, as thermographic records show, the temperature of the air varied considerably in accordance with changing outdoor environmental conditions.

In some experiments the seed alone was treated; in others, the soil alone; and in still others, both the seed and the soil. Counts were made daily and emergence was expressed as the number of seedlings that appeared above ground in relation to the number of seeds planted. The difference between the number of seedlings that emerged and the known germinating capacity of the seed was regarded as indicating fairly closely the reduction in stand due to pre-emergence damping-off. Incidence of postemergence blackroot was determined numerically by expressing the number of typically infected seedlings as a percentage of the number that emerged from the soil. Experiments were allowed to run five weeks, at the end of which time roots of seedlings were given a final examination.

### *Seed*

Segmented seed designated as U.S. 215 × 216 was used throughout the course of the experiments. This seed, furnished by the Canada and Dominion Sugar Company, Chatham, Ont., was representative of warehouse stock that was being distributed by the company to Ontario growers. A first lot of seed obtained in 1945, and used in the 1946-47 tests, had a germinating capacity of 98%. A second lot obtained in 1947, and used in the 1948 tests, had a germinating capacity of 109%. The 1947 seed was noticeably larger than that obtained in 1945, and, in germination tests, the former was found to produce a higher proportion of 'doubles' than did the latter. To ensure as uniform germination as possible, seed to be used in a given test was examined under a dissecting microscope and off-size as well as nonviable-appearing particles

were discarded. In the flats, the seeds, spaced one-half inch apart, were always planted 44 to the row, with three to five equally-spaced rows per flat. In both flats and Wisconsin tank cannisters, the seeds were planted $\frac{1}{2}$ to $\frac{5}{8}$ in. deep.

*Soil*

In all the preliminary and in most of the later experiments, the soil used was a Brookston clay loam obtained from fields where, after repeated failures due to blackroot (acute phase), attempts to grow sugar beets had been abandoned. Mortality of seedlings grown in this soil, even with a high-phosphate commercial fertilizer added, was always high, the postemergence phase of the disease under conditions of moderate watering causing on the average nearly 50% loss of seedlings. Repeated isolations from blackroot-infected seedlings grown in this soil yielded principally cultures of *Aphanomyces cochlioides* Drechs.

As the investigations progressed, the trials were extended to include two other quite different types of soil in which, also, repeated loss of seedlings had been sustained. One of these was a light sandy loam (hereinafter referred to as 'Exeter' sand), and the other, a dark-colored clay of the Clyde series. Blackroot-infected seedlings in the 'Exeter' sand invariably yielded cultures of *Pythium aphanidermatum* (Edson) Fitzpatrick, while from those grown in the Clyde clay, *A. cochlioides* and *Pythium ultimum* Trow were isolated in the ratio of 17 to 5, respectively.

*Fertilizer*

To all flats or containers except those serving as untreated checks, a 2-16-(8, 10, or 6) commercial fertilizer was added at the rate of either 300 or 400 lb. per acre. Half of the fertilizer was placed in a band $1\frac{1}{2}$ in. below the seed, the other half either in contact with the seed, or on the surface of the soil in which latter case it was worked into the top inch of soil before the planting of the seed.

*Protectants*

Although, as previously stated, most of the investigational work herein reported centers around trials in which tetramethylthiuram disulphide was used, nevertheless, in conjunction with these experiments, a number of other materials were subjected to careful test. These proprietary compounds, provided gratuitously by the manufacturers or distributors indicated below, included the following:

*Arasan*—Tetramethylthiuram disulphide, 50% active material, nonwettable; Canadian Industries Limited, Chatham, Ont.

*Tersan*—Tetramethylthiuram disulphide, 50% active material, wettable; Canadian Industries Limited, Chatham, Ont.

*Fermate*—Ferric dimethyldithiocarbamate, 70% active material, wettable; Canadian Industries Limited, Chatham, Ont.

*9-B*—Zinc 2,4,5-trichlorophenate; Dow Chemical Co., Midland, Mich.

*C-119*—Copper trichlorophenate, 20%; Dow Chemical Co., Midland, Mich.

*CCH355, 356, and 358 or 358A*, respectively; Dow Chemical Co., Midland, Mich.

*F800*—2,4,5-trichlorophenyl chloroacetate, 50% active material; Dow Chemical Co., Midland, Mich.

*Mycotox No. 1*—2,4,5-trichlorophenyl acetate; Givaudan-Delawanna Inc., New York, N.Y.

*Cupferron*—Ammonium nitrosophenylhydroxylamine; Eastman Kodak Co., Rochester, N.Y.

*D-419*—Tetramethylthiuram disulphide, 50% active material, nonwettable; Naugatuck Chemical Division, U.S. Rubber Co., Elmira, Ont.

*Dithane D14*—Disodium ethylene bisdithiocarbamate hexahydrate, 25%; P. N. Soden Co., Toronto, Ont.

*Dithane Z-78*—Zinc ethylene bisdithiocarbamate, 65%; P. N. Soden Co., Toronto, Ont.

*Procedure*

The protectants, either alone or mixed with fertilizer, were added to the soil in two ways, which, for convenience of description, may be termed the precision and the surface methods of application.

(*a*) *The precision method.*—A 1-in.-deep layer of the infested field soil was spread uniformly in a flat, fertilizer was spread in the requisite number of bands, and then another inch of soil was added. Thin strips of wood, $22 \times 1\frac{1}{4} \times \frac{1}{4}$ in., were affixed in pairs to delimit 2-in.-wide enclosures running lengthwise of the flat. Meanwhile, a quantity of sieved rootrot soil had been brought to an almost air-dry condition by exposure on a greenhouse bench. To an amount of this soil just sufficient to fill the space enclosed by the wooden frame, *i.e.*, 55 cu. in., the protectant was added at the desired rate, the soil and chemical then being shaken together in a flask. After thorough mixing, the contents of the flask were used to fill the $22 \times 2 \times 1\frac{1}{4}$-in. strip delimited by the wooden frames. The method has been graphically illustrated in an earlier publication (6). Calculations for the amounts of both protectant and fertilizer were made on the basis of the sugar-beet rows in the field being 22 in. apart, *i.e.*, 23,760 linear feet of row per acre. Thus, to determine the quantity of protectant required for a row application in a flat, at a rate, say, of 3 lb. per acre, it was only necessary to calculate the fraction necessary for 22 in., on the basis that in actual field practice 3 lb. would be distributed over 23,760 linear feet.

(*b*) *The surface application method.*—On an inch of rootrot soil in the bottom of a flat, the fertilizer was spread in bands in accordance with the number of rows of seeds to be planted. Then, two additional inches of the rootrot soil were added. Meanwhile, the amounts of the protectant and the fertilizer, determined as above for row application, were thoroughly mixed.

The mixture, having been uniformly spread over the surface of the 2-in.-wide strips where the seed was to be planted, was then worked thoroughly into the top 1¼ in. of soil. The seeds were planted in the center of the treated strips at a depth of about ½ in.

## Investigational

(*a*) *Preliminary Tests*

Of the various protectants listed above, Arasan, fortunately, was one of the first to be investigated. In the earliest exploratory tests, this compound at the rate of 200 lb. per acre was applied to naturally-infested soil by the precision method described above. Inhibition of germination and extreme toxicity to seedlings followed this rate of application. In several series of subsequent tests, the amount of Arasan was scaled down through a range of descending rates of application per acre as follows: 150, 125, 100, 75, 50, 25, 15, 10, 5, 3, and 1 lb., respectively. In all these tests a 2-16-10 commercial fertilizer was applied at the rate of 300 lb. per acre. In one series, the seed but not the soil (except for the addition of fertilizer) was treated with Arasan at the rate of ½ lb. of the protectant per 100 lb. of seed. In another series, untreated seed was planted in lots of the same soil, with and without the addition of fertilizer. Four flats comprised a test unit for each treatment. These various trials were carried out during January, February, and March, 1946. The results obtained are summarized in Table I.

TABLE I

EFFECT OF TREATMENT OF SEED, OF SOIL, AND OF SEED AND SOIL WITH ARASAN, ON EMERGENCE OF SEEDLINGS AND INCIDENCE OF BLACKROOT

| Seed | Treatment | | Emergence of seedlings, %* | Degree of toxicity to seedlings | Incidence of blackroot, %* | |
|---|---|---|---|---|---|---|
| | Soil | | | | | |
| | Arasan | Fertilizer (2–16–10) | | | Pre-emergence phase (approx.)** | Post-emergence phase |
| ½ % Arasan | 15 lb.*** | 150/150 lb.*** | 80.3 | Severe | 17.7† | 0 |
| " | 10 " | " | 89.3 | " | 8.7† | 0 |
| " | 5 " | " | 84.0 | " | 14.0† | 0 |
| " | 3 " | " | 97.7 | Slight | 0.3 | 6.2 |
| " | 1 " | " | 94.1 | None | 3.9 | 15.2 |
| " | Nontreated | " | 95.4 | " | 2.4 | 20.8 |
| Nontreated | " | " | 96.2 | " | 1.8 | 26.7 |
| " | " | Nontreated | 93.9 | " | 4.1 | 42.7 |

* *Average of four flats.*

** *Obtained in this and succeeding tables by subtracting figures for emergence from germinating capacity of seed i.e., 98%.*

*** *Per acre application, the numerator–denominator designation in this and succeeding tables indicating that half the fertilizer was applied with, the other half, below the seed.*

† *These values represent losses due more probably to toxic effects than to parasitic attack.*

As Table I indicates, no blackroot occurred when Arasan was applied at the rates of 15, 10, and 5 lb. per acre. At these concentrations, however, not only was emergence reduced but also severe toxicity symptoms were produced both underground and aboveground on the seedlings. Arasan at 3 lb. per acre had no deleterious effect on germination, induced only scarcely perceptible toxicity symptoms, and inhibited blackroot to the extent of all but 6.2%. At this rate of application, the disease did not appear until the 22nd day after planting. At the 1 lb. per acre application, blackroot developed to the extent of 15.2%, the first appearance of the disease having been noted on the seventh day after planting. When the seed alone was treated, incidence of the postemergence phase of the disease reached 20.8%, thus indicating in these, as in previous trials (7), the ineffectiveness of seed treatment. Highest incidence of blackroot, *i.e.*, 42.7%, occurred when neither seed nor soil was treated with Arasan and when, too, fertilizer was omitted. It will be noted that the addition of fertilizer to the soil reduced the incidence of the postemergence phase of the disease by about 16%. Even in the untreated soil, the pre-emergence phase of the disease was in these tests of only slight significance.

(*b*) *Confirmatory Tests*

Following the preliminary trials described above, many confirmatory tests have been carried out in which attention was concentrated on the 3 or 4 lb. to the acre applications of Arasan. One such test was completed in May–June, 1946, under summer greenhouse conditions. In this test, the Arasan was applied at the rate of 4 lb. per acre. Another test was completed in December, 1946—January, 1947, in the same location but under winter conditions,* the Arasan in this case being applied at the rate of 3 lb. per acre. In both of these tests, the Arasan and the commercial fertilizer were applied by the precision method and four flats comprised the test unit for each treatment and check. Details in connection with these particular trials together with results obtained are recorded in Table II.

As Table II shows, only a negligible percentage of blackroot occurred when the seed and soil, or the soil alone had been treated with Arasan. Thus, the results closely confirmed those obtained in the preliminary trials. While seed treatment was undoubtedly effective in reducing pre-emergence damping-off, nevertheless, such treatment afforded only slight protection against the postemergence phase of the disease. In the nontreated checks, the incidence of postemergence blackroot was 27.1% higher in the summer experiment than in the one completed during the winter months. This higher incidence of the disease is probably to be correlated with the consistently higher soil temperature that prevailed throughout the duration of the summer experiment. In the nontreated checks, it will be noted also that pre-emergence damping-off reduced emergence to the extent of about 22% in both the

* *Representative flats from this test were on display at the meetings of the American Society of Sugar Beet Technologists, held in Detroit, January, 1947.*

TABLE II

EFFECT OF TREATMENT OF SEED AND OF SEED AND SOIL WITH ARASAN ON EMERGENCE OF SEEDLINGS AND INCIDENCE OF BLACKROOT

| Time of experiment | Treatment | | | Emergence of seedlings, %* | Incidence of blackroot, %* | |
|---|---|---|---|---|---|---|
| | Seed | Soil | | | Pre-emergence phase (approx.) | Post-emergence phase |
| | | Arasan | Fertilizer (2-16-10) | | | |
| May–June 1946 | ½ % Arasan | 4 lb.** | 200/200 lb.** | 96.0 | 2.0 | 1.0 |
| | Nontreated | " | " | 91.8 | 6.2 | 2.0 |
| | ½ % Arasan | Nontreated | " | 96.3 | 1.7 | 53.8 |
| | Nontreated | " | " | 76.1 | 21.9 | 65.0 |
| Dec. 1946–Jan. 1947 | ½ % Arasan | 3 lb. | 200/200 lb. | 87.0 | 11.0 | 0.6 |
| | Nontreated | Nontreated | " | 75.3 | 22.7 | 37.9 |

* *Average of four flats.*

** *Per acre applications.*

summer and the winter tests. In Fig. 1, the difference between nontreatment of the soil and treatment with Arasan at 4 lb. per acre are clearly shown, the two series of flats illustrated being taken from the May–June, 1946 experiments.

(*c*) *Supplementary Tests*

While the experiments described above had furnished considerable evidence as to the effectiveness of Arasan in controlling blackroot, nevertheless, many points in connection with the use of this protectant required further investigation. One of the most important of these centered around the possibility of simplification of the method of application. Up to this time, the protectant and the fertilizer had been added separately by the impractical and time-consuming precision method. If the fungicide and the fertilizer could be mixed and the mixture applied to the soil without diminution of effectiveness, an improvement would be effected. The reports of Martin (10) in 1933, of Cunningham (2) in 1936, of Cunningham and Wessels (3) in 1939, and, more recently, of Doran (4, 5), all established precedent for supposing that a fungicide–fertilizer mixture might be used with success in the present problem. If, in addition, the mixture could be applied to the soil in a manner more closely approaching that employed in actual field practice, the mechanics of control by this means would be reduced to a still more practical basis. Of importance, too, from the practical standpoint would be any information that could be gained about the protectant in regard to (*a*) its proper placement in the soil with reference to the seed, (*b*) the length of time it might remain effective in the soil, and (*c*) how its efficacy might be modified by soils of different type and by temperature and moisture of the soil. In addition, it was realized that a continuous effort should be maintained to find, if possible,

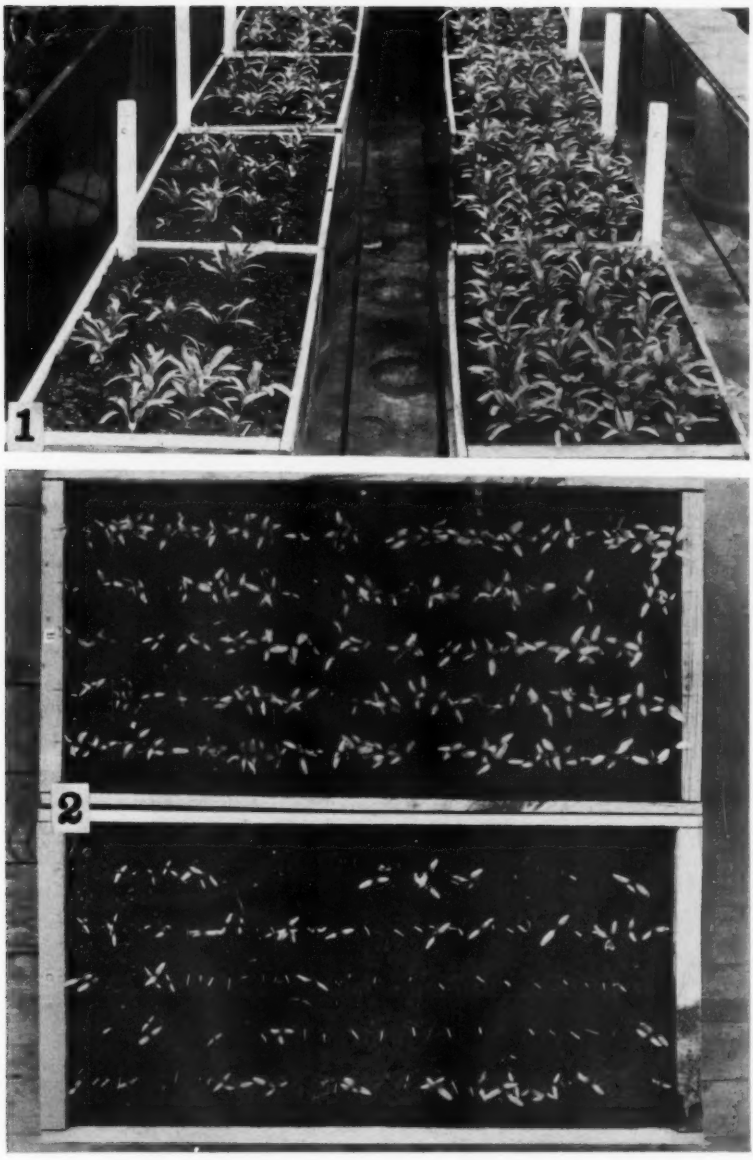



FIG. 1. *Incidence of blackroot in relation to soil treatment with Arasan. All flats received 2-16-10 commercial fertilizer at 400 lb. per acre. Flats to right received, in addition, Arasan at 3 lb. per acre applied by the precision method.* FIG. 2. *Effect of placement of Arasan on incidence of blackroot. In the top flat, the Arasan–fertilizer mixture was mixed with the top 1¼ in. of soil; in the lower one, the mixture was placed in the soil at a depth of 1¼ in., the seed in both cases being planted ¼ to ¾ in. deep.*

FIGS. 3 and 4. *Long-lasting effectiveness of Arasan treatment of soil in controlling blackroot.* FIG. 3. *Surface-method treatment of flats, left to right, respectively, check, commercial fertilizer, fertilizer plus Arasan 3 lb. per acre, and fertilizer plus Arasan 5 lb. per acre. These representative flats photographed Feb. 14, 1948, 16 days after treatment of soil and first planting of seed on Jan. 29.* FIG. 4. *Same flats as above, replanted without further treatment, Mar. 4, and rephotographed Mar. 20, 1948. Thus, after 51 days, Arasan treatment is still highly effective. (Surface of soil covered with thin layer of white quartz sand for better photographic effects.)*

other formulations containing tetramethylthiuram disulphide, or other compounds that might compare favorably with Arasan. With these considerations in mind, the various series of tests described below were planned and carried out.

*I. Compatability of Arasan with Commercial Fertilizer*

Early in January, 1947, Canadian Industries Limited, Chatham, Ont., in anticipation of a future demand for a fertilizer with fungicidal properties, added Arasan to a 2-12-6 commercial fertilizer at the rate of 1 lb. of the former to 99 of the latter, the mixture then being placed in the company's storage bins. As prearranged, after intervals of 1, 2, 4, and 14 months, respectively, samples of the stored mixture were sent to the Harrow laboratory, where their effectiveness in controlling blackroot was compared with that of similar but freshly prepared mixtures of the fungicide and fertilizer. In order to obtain a 3 lb. per acre concentration of Arasan, it was necessary to apply the mixture at 300 lb. per acre. This meant a much heavier application of the fertilizer component than was employed in other tests. The experiments, carried out, as they were, with various periods of time intervening, had necessarily to be conducted under conditions that varied as to temperature and light intensity. The mixture was in all cases applied by the precision method and, as usual, four flats comprised a test unit for each treatment and check. In none of the tests of this series was the seed treated. In the last series of tests, *i.e.*, the series involving the 14-month-old mixture, 1947 seed instead of 1945 seed had to be used, since the supply of the latter had become exhausted. The soil used was the Brookston clay loam. Results obtained as well as other details in connection with these several tests are assembled in Table III.

The points of outstanding interest in the results shown in Table III are that (*a*) after being mixed with the fertilizer for as long as 14 months, Arasan had lost little of its fungicidal properties, and (*b*) the mixture of Arasan and fertilizer of various ages up to 14 months, though perhaps perceptibly less effective in controlling the disease than the freshly prepared mixture, was not significantly so. Such evidence would seem to be sufficient to establish the fact of compatability between the fungicide and fertilizer over a relatively long period of time. Commercial firms would thus be afforded ample opportunity of preparing mixtures of the two well in advance of when they might be required.

Table III shows that, in soil treated with the fertilizer alone, the incidence of pre-emergence damping-off, ranging as it does from 25.9% in the Feb.–March, 1947 tests to 48.2% in those of Apr.–May, 1948, was higher than might be expected. In this connection it should be pointed out that these and other tests were conducted in a greenhouse in which it was impossible to regulate the temperature other than by opening vents or closing valves. Thus, from mid-March, through April and early May, the sugar-beet flats were often subjected to overhead heat from the sun as well as that arising

TABLE III

EFFECT OF SOIL TREATMENT WITH 1- 2-, 4-, AND 14-MONTH-OLD ARASAN-FERTILIZER MIXTURES AS COMPARED WITH THAT OF SIMILAR BUT FRESHLY PREPARED MIXTURES ON EMERGENCE OF SEEDLINGS AND INCIDENCE OF BLACKROOT

| Duration of experiment | Per acre soil treatment | Emergence, %* | Incidence of blackroot, %* | |
|---|---|---|---|---|
| | | | Pre-emergence phase (approx.) | Post-emergence phase |
| Feb. 3 to Mar. 10, 1947** | 300 lb. 1-month-old (2-12-6 + Arasan) mixture | 87.1 | 10.9 | 6.8 |
| | 300 lb. freshly prepared (2-12-6 + Arasan) mixture | 89.4 | 8.6 | 5.3 |
| | 300 lb. 2-12-6 | 62.1 | 25.9 | 33.9 |
| Mar. 3 to Apr. 7, 1947** | 300 lb. 2-month-old (2-12-6 + Arasan) mixture | 79.5 | 18.5 | 6.6 |
| | 300 lb. freshly prepared (2-12-6 + Arasan) mixture | 87.4 | 10.6 | 4.8 |
| | 300 lb. 2-12-6 | 57.9 | 40.1 | 30.1 |
| May 6 to June 10, 1947** | 300 lb. 4-month-old (2-12-6 + Arasan) mixture | 92.3 | 5.7 | 7.8 |
| | 300 lb. freshly prepared (2-12-6 + Arasan) mixture | 88.6 | 9.4 | 6.1 |
| | 300 lb. 2-12-6 | 43.4 | 44.6 | 37.5 |
| Apr. 6 to May 11, 1948*** | 300 lb. 14-month-old (2-12-6 + Arasan) mixture | 103.3 | 5.7 | 7.4 |
| | 300 lb. freshly prepared (2-12-6 + Arasan) mixture | 107.7 | 1.2 | 5.6 |
| | 300 lb. 2-12-6 | 60.8 | 48.2 | 35.0 |

* *Average of four flats.*

** *1945 and* ****1946 seed, average germinating capacity 98 and 109%, respectively.*

from the heating pipes. Under such conditions, the temperature not infrequently exceeded 100° F. for varying periods of time. This necessitated more frequent watering, with the result, as might be expected, that incidence of pre-emergence damping-off was higher than that ordinarily encountered in the field.

Table III shows also that, in soils treated with the Arasan–fertilizer mixture, incidence of postemergence blackroot ranged from 4.8 to 7.8%. If, as in previous tests, the seed had been treated, the incidence both of post- and pre-emergence damping-off would probably have been appreciably reduced.

### *II. Simplication of the Method of Applying Arasan to the Soil*

Coincidently with the compatability tests described above, attention was being directed towards finding a method of applying the Arasan–fertilizer mixture that could replace the highly impractical precision method. With this in view, a preliminary experiment was carried out in which fertilizer alone, and fertilizer with Arasan added at three different rates, namely, 3, 5, and

7 lb. per acre, respectively, were applied to the soil (Brookston clay loam) by the surface application method. In this experiment, four flats comprised the test unit for each of the four treatments and untreated check. Five rows of untreated 1945 seed, 44 per row, were planted in each flat. Thus, in the 20 flats comprising the test, a total of 4400 seeds were planted. The experiment, begun on Jan. 29, 1948, terminated five weeks later on Mar. 4. The results, as will be noted below, indicated that without any loss of effectiveness the surface application method might readily replace the precision method. However, to check this point more carefully, two additional series of tests, *B* and *C*, were carried out, in which the two methods were compared simultaneously. In these supplementary tests, 1947 instead of 1945 seed had to be used but otherwise the trials were carried out in as exact replication as possible. Details in connection with the three trials, including results obtained, are assembled in Table IV.

TABLE IV

COMPARATIVE EFFECTIVENESS OF PRECISION AND SURFACE-APPLICATION METHODS OF APPLYING ARASAN-FERTILIZER MIXTURE TO SOIL FOR CONTROL OF BLACKROOT

| Duration of experiment | Per acre soil treatment | | Method of application | Emergence, %* | Incidence of blackroot, %* | | Av. no. of surviving seedlings per ft. of row* |
|---|---|---|---|---|---|---|---|
| | Arasan | Fertilizer (2-16-8) | | | Pre-emergence phase | Post-emergence phase | |
| *A*** | 7 lb. | 200/200 lb. | Surface | 79.0 | 18.0 | 2.0 | 18.6† |
| | 5 lb. | " | " | 87.2 | 10.8 | 4.7 | 19.9 |
| Jan. 29 to Mar. 4, | 3 lb. | " | " | 92.3 | 5.7 | 5.4 | 16.8 |
| 1948 | Nil | " | " | 46.3 | 51.7 | 47.6 | 5.7 |
| | Nil | Nil | | 36.7 | 61.3 | 47.4 | 4.6 |
| *B**** | 3 lb. | 200/200 lb. | Precision | 102.6 | 6.4 | 16.8 | 20.3† |
| Apr. 6 to May 11, | 3 lb. | " | Surface | 103.3 | 5.7 | 11.2 | 22.0† |
| 1948 | Nil | " | | 101.9 | 7.1 | 35.0 | 10.1 |
| *C**** | 4 lb. | Nil | Surface | 109.0 | Nil | 7.2 | 24.2 |
| | 4 lb. | 200/200 lb. | Surface | 113.5 | Nil | 2.7 | 26.3 |
| June 15 to July 20, | 4 lb. | " | Precision | 107.6 | 1.4 | 2.6 | 25.0 |
| 1948 | Nil | " | Surface | 70.4 | 43.5 | 31.6 | 11.7 |
| | Nil | Nil | | 92.6 | 16.4 | 55.8 | 9.8 |

* *Average of four flats.*

** *1945 and* ****1947 seed used, average germinating capacity 98 and 109%, respectively.*

† *Toxicity symptoms apparent.*

As shown in Table IV and as illustrated in Fig. 3, the results of Expt. *A* indicated that the surface method of application of the Arasan–fertilizer mixture might be quite as effective as the precision method in controlling blackroot. When Arasan was applied at 5 or 3 lb. per acre by the new method, loss of seedlings due to pre-emergence damping-off was relatively low, while that due to the postemergence phase of the disease did not exceed 5.4%.

These losses are in marked contrast to those recorded for soil that had not been treated or to which only fertilizer had been added. A 7 lb. per acre application of Arasan reduced emergence and induced marked toxicity symptoms on many seedlings.

In Expt. *B*, in which for the first time the two methods were tested simultaneously, the incidence of blackroot was lowest in both phases of the disease, following the surface method of application. It will be noted that, for both methods, the incidence of postemergence blackroot was unusually high. The reason for this was explained in the preceding section, namely, excessively high temperature necessitating more frequent watering during a critical period of the experiment. In this connection, it should be pointed out that on 13 days during the earlier part of the experiment, when the greenhouse had still to be heated artificially, the temperature, for periods varying from one to several hours, ranged from 90° to 100° F., and, on nine days, exceeded 100°, approaching a maximum of 110° on the three successive days (9th, 10th, and 11th days after planting) during which the seedlings were in the most susceptible stage. In this experiment, there were indications also of Arasan injury, as evidenced not only by typical foliar symptoms but also by some burning of the roots. The possibility that, at high soil temperatures, Arasan may prove to be more or less phytotoxic was confirmed by experiments that are described later in the present paper.

In Expt. *C*, as the data indicate, the fact of the surface application method being as efficient as the precision method was confirmed.

In Table IV, as will be noted, a new criterion for evaluating the effectiveness of the addition of Arasan to the soil has been added, namely, a comparison of the number of surviving seedlings per foot of row in treated and nontreated soil. At the very least, the ratio is two to one (Expt. *B*) in favor of the treated soil and is still higher in the other two experiments.

### *III. Correct Placement of Arasan*

Of the many points to be considered in the treatment of soil with Arasan, none is more important, especially from the standpoint of field practice, than that of proper placement of the protectant. As has already been pointed out in the introductory paragraphs, under field conditions, the postemergence phase of the disease is, in general, more to be feared than the pre-emergence phase. In the postemergence phase, the hypocotyl of the seedling is attacked at or near the soil level. Theoretically, it would seem best to so place the Arasan that it would not only surround the seed but would also be incorporated in the soil between the seed level and the surface of the soil. Such placement should afford protection against pre- and postemergence phases of the disease. To test the validity of this postulation, an experiment was carried out in which, in certain flats, by employing the surface method of application, the Arasan–fertilizer mixture was thoroughly mixed in the top $1\frac{1}{4}$ in. of soil (Brookston clay loam). The seed was then planted to a depth

of ½ to ¾ in. In a corresponding number of flats, an Arasan–fertilizer mixture of the same concentration was applied to the soil at a depth of 1¼ in. below the surface, the seed again being planted ½ to ¾ in. deep. The test was repeated and the results of the two trials are recorded in Table V.

TABLE V

EFFECT OF PLACEMENT OF ARASAN IN SOIL ON EMERGENCE OF SEEDLINGS AND INCIDENCE OF BLACKROOT

| Duration of experiment | Per acre soil treatment | | Placement of Arasan | Emergence, %* | Incidence of blackroot, %* | |
|---|---|---|---|---|---|---|
| | Arasan | Fertilizer (2-16-8) | | | Pre-emergence phase (approx.) | Post-emergence phase |
| June 15 to July 20, 1948 | 4 lb. | 200/200 lb. | Top inch of soil | 113.5 | Nil | 2.7 |
| | 4 lb. | " | Depth of 1¼ in. | 62.9 | 46.1 | 40.4 |
| | Nil | " | | 82.3 | 26.7 | 17.0 |
| | Nil | Nil | | 92.6 | 16.4 | 55.8 |
| Aug. 17 to Sept. 21, 1948 | 4 lb. | 200/200 lb. | Top inch of soil | 114.0 | Nil | 1.1 |
| | 4 lb. | " | Depth of 1¼ in. | 77.7 | 31.3 | 21.6 |
| | Nil | Nil | | 58.6 | 50.4 | 46.3 |

* *Average of three flats.*

As Table V shows, when the seed germinated in, and the seedling grew up through soil with which had been incorporated the Arasan–fertilizer mixture, the incidence of disease was virtually negligible. In marked contrast, as is shown in Fig. 2, when the mixture was placed below the seed and the seedling grew up through the soil without the protection of Arasan, the incidence of both phases of damping-off was high. These tests, while admittedly on a limited scale, demonstrate conclusively, nevertheless, not only the importance of adding Arasan to infested soil but also the necessity of so placing it that best results may be obtained. The implications as relating to field practice are of especial importance.

### *IV. Effectiveness of Arasan in Soils of Different Types*

As mentioned in an earlier section, blackroot has been observed to cause striking loss of seedling stands in at least three quite different types of soil, namely, Brookston clay loam, 'Exeter' sandy loam, and dark-colored clay of the Clyde series. It was a matter both of interest and of importance to find out whether and to what extent Arasan might control the disease in these different soils, the more especially since it was known that different pathogens were associated with each. Consequently, using 1945 seed and employing the precision method of application, tests were set up with results as shown in Table VI.

TABLE VI

EFFECTIVENESS OF ARASAN TREATMENT OF SOILS OF DIFFERENT TYPE ON EMERGENCE OF SEEDLINGS AND INCIDENCE OF BLACKROOT

| Soil type | Predominating pathogen(s) | Treatment | | | Emergence, %* | Incidence of blackroot, %* | |
|---|---|---|---|---|---|---|---|
| | | Seed | Soil | | | Pre-emergence phase (approx.) | Post-emergence phase |
| | | | Arasan | Fertilizer | | | |
| Brookston clay loam | *Aphanomyces cochlioides* | ½ % Arasan | 4 lb.** | 200/200 lb.** | 96.0 | 2.0 | 1.0 |
| | | Nil | 4 lb. | " | 91.8 | 6.2 | 2.0 |
| | | Nil | Nil | " | 76.1 | 21.9 | 65.0 |
| 'Exeter' sandy loam | *Pythium aphanidermatum* | ½ % Arasan | 4 lb. | 200/200 lb. | 103.4 | Nil | Nil |
| | | Nil | Nil | " | 63.6 | 34.4 | 69.0 |
| Clyde clay | *A. cochlioides* *Pythium ultimum* | ½ % Arasan | 4 lb. | 200/200 lb. | 102.7 | Nil | Nil |
| | | Nil | Nil | " | 78.4 | 19.6 | 6.5 |

* *Average of four flats.*
** *Per acre applications.*

As Table VI shows, the Arasan treatment was effective in reducing the incidence of the disease in all three types of soil, the more especially so, however, in the case of the Brookston clay and the 'Exeter' sandy loam.

### *V. Comparative Effectiveness of Arasan and Other Formulations Containing Tetramethylthiuram Disulphide as the Lethal Agent*

The only other formulations containing tetramethylthiuram disulphide as the active ingredient that were tested were Tersan and a product of Naugatuck Chemicals, Division of Dominion Rubber Company, Elmira, Ont. This product was labelled "Control D-419, tetramethylthiuram disulphide–50% non-wettable". As contrasted with the pink color of Arasan, D-419 is white and it also has a different odor. Thus, it would appear that the two products might differ as to their inert ingredients. In the tests, Tersan, like Arasan and D-419, was applied in the dry powder form. Details in connection with these tests together with results obtained are assembled in Table VII.

As Table VII shows, the results obtained with Arasan and D-419 were, in the aggregate, appreciably better than those with Tersan, the apparent superiority of the two first-mentioned compounds being more noticeable in the control of the postemergence phase of the disease. According to the results as tabled, there would seem to be little choice between Arasan and D-419. However, certain observations made during the tests reveal some interesting possibilities with respect to D-419. In the Jan. 13 – Feb. 17 test, for example, emergence of seedlings for treatments and checks began on the

TABLE VII

COMPARATIVE EFFECTIVENESS OF TREATMENT OF SEED AND SOIL, OR OF SOIL, WITH ARASAN, TERSAN, AND D-149, ON EMERGENCE OF SEEDLINGS AND INCIDENCE OF BLACKROOT

| Duration of experiment | Treatment | | | Emergence, %* | Incidence of blackroot, %* | |
|---|---|---|---|---|---|---|
| | Seed | Soil | | | Pre-emergence phase (approx.) | Post-emergence phase |
| | | Protectant | Fertilizer (2-16-10) | | | |
| Mar. 3 to Apr. 7, 1947*** | Nil | Tersan, 3 lb.** | 200/200 lb.** | 88.5 | 9.5 | 5.7 |
| | " | Arasan, 3 lb. | " | 90.5 | 7.5 | 0.6 |
| | " | Nil | " | 49.7 | 46.3 | 51.1 |
| Jan. 13 to Feb. 17, 1948*** | Nil | Tersan, 3 lb. | 200/200 lb. | 82.3 | 15.7 | 6.7 |
| | ½ % Arasan | Arasan, 3 lb. | " | 85.9 | 12.1 | 2.7 |
| | ½ % D-419 | D-419, 3 lb. | " | 78.4 | 19.6 | 4.8 |
| | ½ % D-419 | D-419, 4 lb. | " | 82.5†† | 15.5 | 2.7 |
| | Nil | Nil | " | 62.3 | 35.7 | 41.4 |
| June 15 to July 20, 1948† | Nil | Arasan, 4 lb. | 200/200 lb. | 110.5 | Nil | 2.7 |
| | " | D-419, 4 lb. | " | 116.5†† | Nil | 0.5 |
| | " | Nil | " | 82.3 | 26.7 | 17.0 |

* *Average of three flats.*

** *Per acre applications.*

*** *1945 and † 1947 seed used, average germinating capacity 98 and 109%, respectively.*

†† *Toxicity symptoms apparent.*

seventh day after planting the seed. However, first- and second-day emergence for the D-419 treatments was much higher than for those for Arasan or check, as the following figures disclose:

| Treatment | Emergence, days after planting, % | |
|---|---|---|
| | Seventh day | Eighth day |
| Arasan, 3 lb. | 1.5 | 18.4 |
| Check | 1.3 | 17.1 |
| D-419, 3 lb. | 9.5 | 50.7 |
| D-419, 4 lb. | 7.5 | 46.2 |

It was also observed subsequently that the seedlings in the soil receiving the 3 lb. per acre application of D-419, were outstanding in vigor of growth. In the flats receiving the 4 lb. per acre application of D-419, many of the seedlings exhibited toxicity symptoms that corresponded in severity with those on seedlings grown in soils that had received slightly heavier applications of Arasan. From these observations it would appear that D-419 is slightly 'stronger' than Arasan. Since, however, each contains 50% of the active ingredient, any difference in 'strength' would seem to be inherent in the 'inert' ingredients. In any case, the efficacy of D-419 was such as to warrant rating it at the very least on a par with Arasan.

### *VI. Period of Time During Which Arasan Remains Effective in the Soil*

Often, under field conditions, a period ensues just after planting, when, as the result of unfavorable environmental conditions, germination or emergence is delayed and the seeds or seedlings need added protection against the soil-borne pathogens. Under such adverse conditions, the prime requisite of a protectant is that its protective action be extended over a long enough period of time during the critical period to ensure an adequate stand of seedlings. In order to gain some information as to how long Arasan might remain effective in the soil, a few exploratory experiments were carried out. In an earlier experiment in which 1945 seed was used, the Arasan was applied by the precision method, three weeks prior to planting, to lots of Brookston clay soil that meanwhile were kept wet and dry, respectively. In a later experiment, at the conclusion of a routine five-week test in which the Arasan had been applied by the surface method of application, the seedlings were removed and, with no additional treatment of the soil, a replicate planting was made by pushing the seeds to a depth of $\frac{5}{8}$ in. in the soil, along the original rows. Details in connection with these experiments together with results obtained are recorded in Table VIII.

TABLE VIII

EFFECT OF PREPLANTING TREATMENT OF SOIL WITH ARASAN, ON EMERGENCE OF SEEDLINGS AND INCIDENCE OF BLACKROOT

| Expt. | Soil treatment | | | | | Emergence, %* | Incidence of blackroot, %* | |
|---|---|---|---|---|---|---|---|---|
| | Arasan application | | | Moisture condition of soil prior to application | Fertilizer (2–16–10) | | Pre-emergence phase (approx.) | Post-emergence phase |
| | Rate | Method | Time | | | | | |
| I*** | 3 lb.** | Precision | Three weeks before planting | Dry, 3 weeks | 200/200 lb.** | 91.7 | 6.3 | |
| | 3 lb. | " | " | Mod. wet, 3 weeks | " | 86.0 | 14.0 | |
| | 3 lb. | " | At planting | Dry, 3 weeks | " | 100.6 | Nil | |
| | 3 lb. | " | " | Mod. wet, 3 weeks | " | 100.6 | Nil | |
| | Nil | | | | " | 60.2 | 27.8 | |
| | 5 lb. | Surface | Five weeks before planting | Mod. wet, 5 weeks | 200/200 lb. | 116.3 | Nil | 2.9 |
| II† | 3 lb. | " | " | " | " | 115.2 | Nil | 6.8 |
| | Nil | | | | " | 71.0 | 38.0 | 23.6 |
| | Nil | | | | Nil | 62.4 | 36.6 | 38.2 |

* *Average of four flats.*

** *Per acre applications.*

*** *1945 and † 1947 seed, average germination 98 and 109%, respectively.*

In Expt. I, as Table VIII shows, Arasan applied at planting time afforded more complete protection against the disease than when applied three weeks prior to planting. In the wet soil the incidence of disease was appreciably

higher than in the corresponding dry soil. The results of Expt. II are especially interesting. After effectively protecting an earlier stand of seedlings (see Table IV, Expt. *A* and Fig. 3), the Arasan at both 5 and 3 lb. per acre, afforded, virtually, equally as high protection to those of the replicate planting (see Fig. 4). The latter was made on Mar. 4, 1948. First emergence was noted on Mar. 9 and the period of highest seedling mortality was from Mar. 11 to 14, inclusive. The Arasan had been originally applied on Jan. 29. Thus, after a period of at least 45 days, the effect of Arasan had not been dissipated. These experiments, while admittedly limited both as to number and scope, indicate clearly, nevertheless, that Arasan may be expected to be effective over relatively long periods of time.

*VII. Effect of Temperature and Moisture on Arasan-treated Soil*

All the experiments described above had been carried out under conditions that in a number of instances permitted relatively wide variations as to both temperature and moisture of the soil. In certain of the trials, it had been indicated that each of these variables—more particularly temperature, perhaps—had not been without effect in modifying either the fungicidal or the phytotoxic possibilities of Arasan. Under these circumstances, it seemed advisable to test the protectant under conditions of more precise control of the two variables in question. For such tests there were available three Wisconsin temperature tanks with cannisters 18½ in. deep and 7¾ in. in diameter. A cannister was prepared as follows. First, two flower pots, each of 6½ in. depth and 6½ in. diameter, one inverted over the other, were set in a cannister. Then, sufficient gravel to fill in around the pots and to cover them to a depth of ½ in. was added. Meanwhile, a quantity of the naturally-infested Brookston clay soil had been sieved through a screen of ⅜ in. mesh. A 2 in. layer of this soil was placed on top of the gravel. An amount of fertilizer representing one-half of the quantity to be used was then applied and an inch of Arasan-treated rootrot soil was added. The second half of the fertilizer was then applied and, lastly, another inch of the Arasan-treated soil added, the top of the latter being 1¾ in. below the rim of the cannister. Seed (1945) to the number of 60 per cannister was pushed to a depth of ⅝ in. in the top layer of the treated soil. Eight cannisters were prepared for each tank in this way, except that in the four that were to serve as checks, the rootrot soil was not treated with Arasan. The amounts of both Arasan and fertilizer requisite for the cannister type of container were carefully calculated from the data available from the many previous experiments. One of the three tanks was operated at 14° to 16° C., another at 19° to 21° C., and the third at 27° to 29° C. An attempt was made to maintain the soil in all cannisters at a uniformly moderate level of moisture. To do this, it was found necessary to add 150 cc. of water three times a day to each cannister of the high temperature series, 50 cc. once daily to each of those of the intermediate temperature, and smaller amounts at longer intervals to the low temperature one. Two tests, one beginning Nov. 20, 1947, the other Jan. 12, 1948, were completed. The same

technique was used in both but, in the second, the seed was treated as well as the soil, the amount of Arasan added to the soil being at the rate of 3 lb. per acre. Details in connection with these tests together with the results obtained are assembled in Table IX.

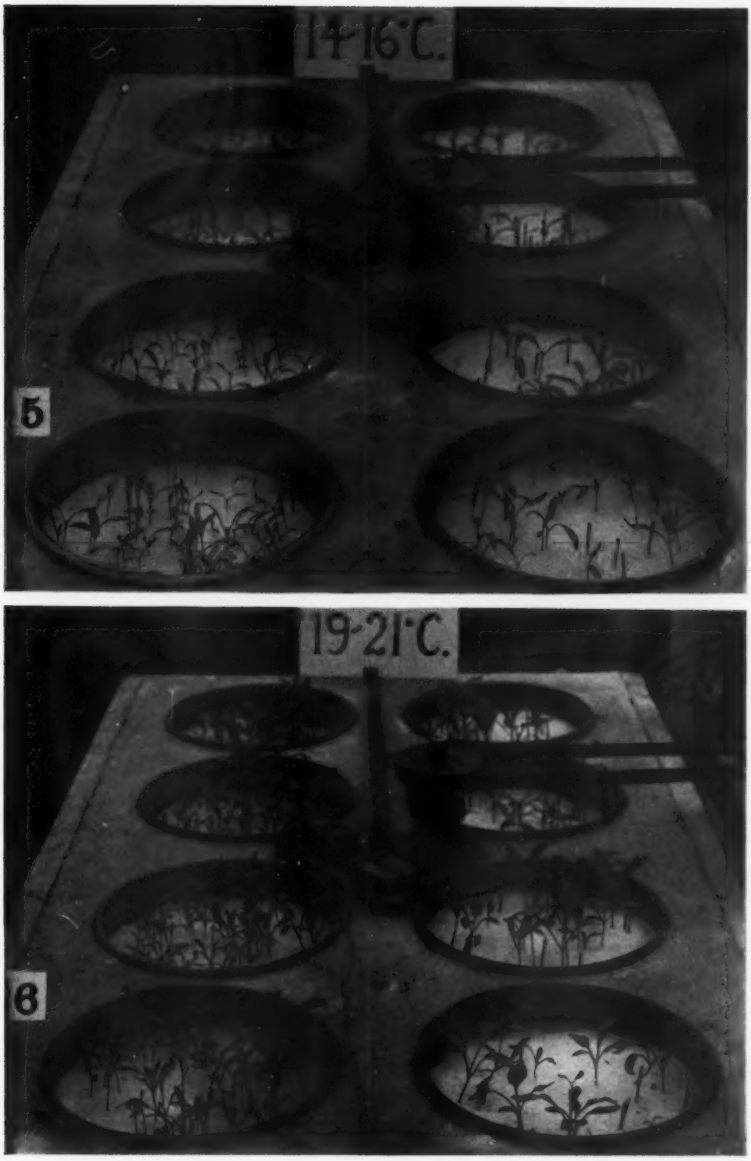
TABLE IX

EFFECT OF TEMPERATURE AND MOISTURE ON ARASAN-TREATED SEED AND SOIL, AS INDICATED BY EMERGENCE OF SEEDLINGS AND INCIDENCE OF BLACKROOT

| Soil temperature | Expt. | Treatment | | | Emergence, %* | Arasan toxicity | Incidence of blackroot, %* | |
|---|---|---|---|---|---|---|---|---|
| | | Seed | Soil | | | | | |
| | | | Arasan | Fertilizer (2-16-10) | | | Pre-emergence phase (approx.) | Post-emergence phase |
| 27° to 29° C. | 1 | Nil | 4 lb. | 200/200 lb. | 87.9 | Severe | 10.1 | |
| | 2 | ½ % Arasan | 3 lb. | " | 85.7 | Moderate | 12.2 | 2.9 |
| | 1 | Nil | Nil | " | 57.9 | | 40.1 | 32.4 |
| | 2 | Nil | Nil | " | 61.2 | | 36.8 | 25.8 |
| 19° to 21° C. | 1 | Nil | 4 lb. | " | 87.9 | | 10.1 | 4.2 |
| | 2 | ½ % Arasan | 3 lb. | " | 75.0 | | 23.0 | 3.3 |
| | 1 | Nil | Nil | " | 43.7 | | 54.3 | 31.4 |
| | 2 | Nil | Nil | " | 62.9 | | 35.1 | 19.2 |
| 14° to 16° C. | 1 | Nil | 4 lb. | " | 91.2 | | 7.8 | 4.1 |
| | 2 | ½ % Arasan | 3 lb. | " | 84.5 | | 13.5 | 0.4 |
| | 1 | Nil | Nil | " | 47.0 | | 51.0 | 24.7 |
| | 2 | Nil | Nil | " | 47.9 | | 40.1 | 13.0 |

* *Average of four cannisters.*

So far as the main interests of this paper are concerned, the most important point, as revealed by Table IX and as clearly indicated in Figs. 5 and 6, is that, up to a temperature of 21° C. (= 70° F.), at least, Arasan applied at a rate as high as 4 lb. per acre will, in a moderately wet soil, effectually control blackroot, without injury to the seedlings, the extent of control of the post-emergence phase of the disease under these circumstances being especially noteworthy. At 27° to 29° C., moderate injury resulted from a 3 lb. per acre application of Arasan to the soil. This injury, scarcely discernible on the foliage, appeared as a burning of the roots and lower hypocotyl, affected tissues also exhibiting a tendency towards flaccidity. As a result of the 4 lb. per acre application of the protectant, the types of injury just described were accentuated. In addition, typical foliar symptoms were noted, the injury in a number of seedlings being so severe as to cause their death. That the types of injury observed could in no way be ascribed to the fertilizer was proved by the fact that the roots of the surviving seedlings in the similarly fertilized check soil were turgid and healthy. That Arasan will injure the roots of seedlings if the temperature is high enough is a point of academic




FIGS. 5 and 6. *Effectiveness of Arasan treatment under conditions of controlled temperature and moisture of the soil. All cannisters received 2-16-10 commercial fertilizer at 400 lb. per acre. Cannisters to left received in addition Arasan at 4 lb. per acre applied by the precision method. (Surface of soil covered with thin layer of white quartz sand for better photographic effects.)*

interest rather than one of practical significance, because rarely, if ever, would the temperature of the soil at the time that sugar beets are normally planted reach or remain long enough at the danger point for injury to take place.

*Other Proprietary Materials in Comparison with Compounds Containing Tetramethylthiuram Disulphide*

In the present studies, some 11 other proprietary materials in addition to Arasan, Tersan, and D-419, were tested as to their effectiveness in controlling blackroot. While some of these were found to be totally inadequate for the purpose, certain others, including Mycotox No. 1 (2, 4, 5-trichlorophenyl acetate), C-119 (copper trichlorophenate, 20%), CCH-358 or 358A, and F-800 (2,4,5-trichlorophenyl chloroacetate, 50%)—the three latter supplied by the Dow Chemical Company—showed encouraging possibilities. C-119 gave especially good results in greenhouse experiments but, when tested later under field conditions, failed to afford adequate protection to the seedlings. Under cooler greenhouse conditions, CCH-358 or 358A, and F-800 compared favorably with Arasan, but, when the tests were repeated under higher temperature conditions, their effectiveness was greatly diminished. In regard to these products, there is some evidence to suggest that they are fungistatic rather than fungicidal in their capabilities.

## Discussion

Since blackroot of sugar beets is so essentially a field problem, methods and materials used in greenhouse investigations, such as those recorded above, must be evaluated primarily from the standpoint of the possibility of their adaptability to field practice under field conditions. Moreover, in consideration of the extremely wide difference in precision that must necessarily exist between field and greenhouse methods, the results obtained in greenhouse experiments must be of such a convincing nature as to indicate the probability of a reasonable measure of success from analogous field experiments. If the materials and methods employed in the experiments described in this paper, as well as the results obtained, are examined in the light of the above considerations, there would seem to be suggested a particularly promising avenue of approach to the remedying or control of the disease in question. In the first place, the question of the materials involved offers no serious problem to those concerned. Proprietary materials, such as Arasan or D-419, containing the necessary active agent, tetramethylthiuram disulphide, are readily available to the trade and can be mixed with commercial fertilizers well in advance of the time the mixture may be required for use. Since no appreciable benefit is to be derived from seed treatment, that process can be eliminated. The grower receives the fungicide–fertilizer mixture all ready for use. Many growers already possess—or can be furnished with—the type of sugar-beet machinery with which the mixture can be successfully applied, provided that

care is exercised as to correct rate of application and proper placement of the protectant.* The row application method and the relatively small amount of the protectant required would make it economically possible for almost any grower to reap advantage of the benefits to be derived from the treatment. Even if the sugar-beet seed is delayed in germination, the grower may still expect the seedlings to be protected, since Arasan, for example, has been found to remain effective in the soil over relatively long periods of time. In fact, this is probably one of the more important reasons why the materials containing tetramethylthiuram disulphide proved to be so much more effective than many of the other materials tested. Admittedly, under certain combinations of circumstances, the treatment may be expected to lose some of its effectiveness. If, for example, when seedlings are in the stage at which they are most susceptible to the disease, the temperature and moisture of the soil should both be excessively high—more particularly the temperature, perhaps—then, the treatment may not afford sufficient protection to ensure an adequate and uniform stand of seedlings. Such a combination of unfavorable circumstances would be more likely to be encountered in late plantings of sugar beets.

When the data tabulated above are examined and compared, it will be found that, invariably, regardless of experimental procedure, emergence of seedlings was greater and incidence of blackroot was lower in Arasan-treated than in nontreated rootrot soil, the difference resulting from treatment with the chemical being so great as to indicate significance without recourse to statistical analysis. The differences were of such a consistent and decisive nature as to suggest also the high probability of success of the methods and materials involved when applied in field practice.

As a recent report by Leach and Snyder (9) suggests, workers elsewhere are becoming interested in the possibilities of localized chemical applications to the soil for the control of soil-borne diseases of crops other than sugar beets.

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## References

1. COONS, G. H., KOTILA, J. E., and BOCKSTAHLER, H. W. Blackroot of sugar beets and possibilities for its control. Proc. Am. Soc. Sugar Beet Technol. 364-380. 1946.
2. CUNNINGHAM, H. S. The addition of mercury compounds to the fertilizer mixture as a control for common scab of the potato under Long Island conditions. Am. Potato J. 13 : 100-103. 1936.

* *This statement is based on the results of field experiments that have already been carried out but an account of which will be reserved for a complementary paper.*

3. CUNNINGHAM, H. S. and P. H. WESSELS. Controlling common scab of the potato on Long Island by the addition of mercury compounds to the fertilizer mixture and the relation of soil reaction to the treatment. N.Y. Agr. Expt. Sta. Bull. 685. 1939.
4. DORAN, W. L. Diseases of plants caused by soil-infesting organisms, with particular attention to control measures. Mass. Agr. Expt. Sta. Bull. 428. Ann. Rept. p. 21. 1944-45.
5. DORAN, W. L. Fungicides applied in fertilizer for the control of cabbage clubroot and damping-off. Phytopathology, 37 : 848. 1947.
6. HILDEBRAND, A. A. The efficacy of soil applications of fungicides for the control of blackroot of sugar beet seedlings. Proc. Am. Soc. Sugar Beet Technol. 28-30. 1947.
7. HILDEBRAND, A. A. and L. W. KOCH. Studies on blackroot of sugar beet seedlings. Sci. Agr. 23 : 557-567. 1943.
8. HULL, RAYMOND. Fungi, viruses and sugar. Presidential address, Lincolnshire Naturalists' Union, pp. 1-14. 1945.
9. LEACH, L. D. and W. C. SNYDER. Localized chemical applications to the soil and their effects upon root rots of beans and peas. Phytopathology, 37 : 363. 1947.
10. MARTIN, WM. H. Fertilizer–mercury combination for the control of scab and *Rhizoctonia*. Hints to potato growers, 18 (8). 1933.

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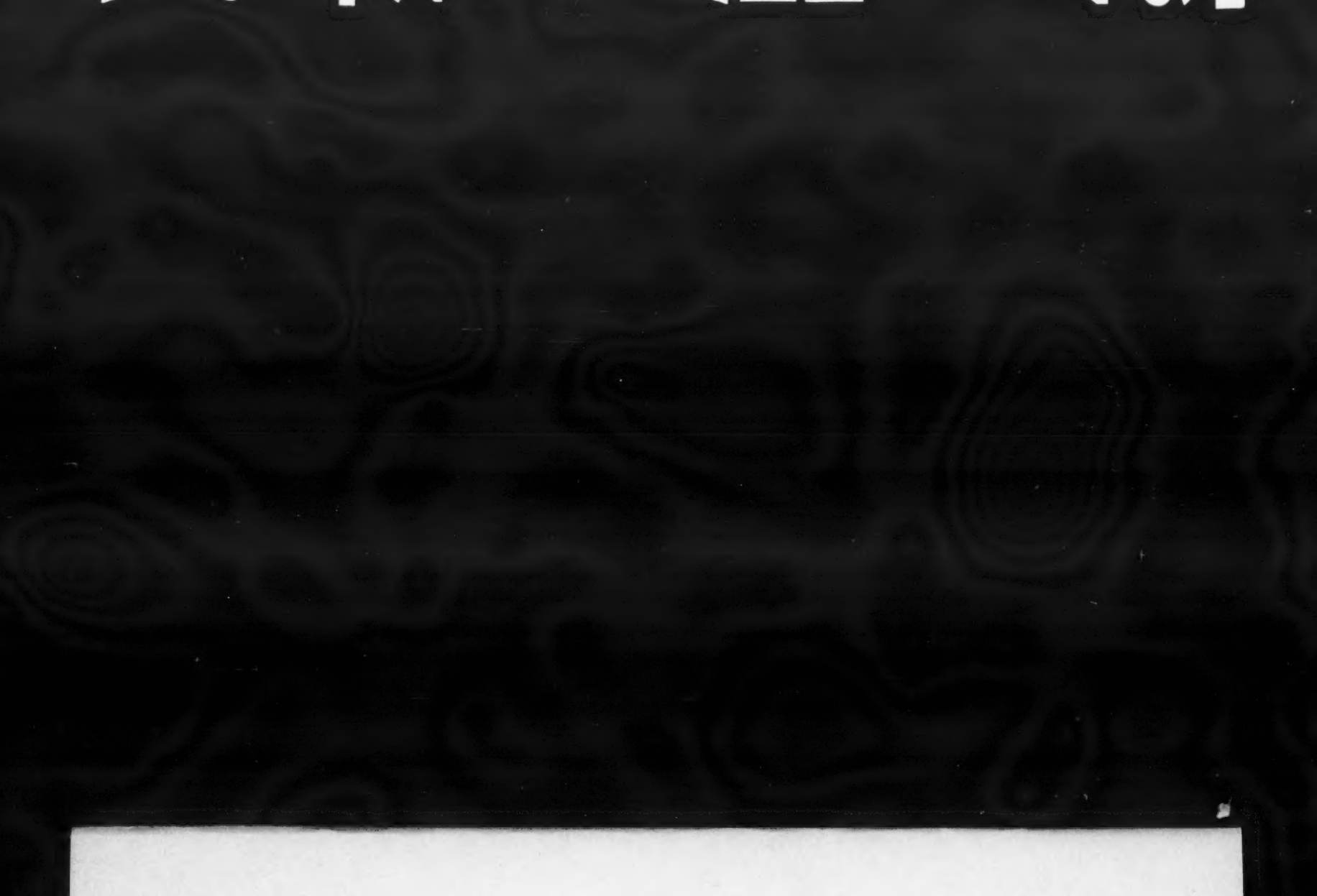
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